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United States Patent [19]

Weiss et al.

[11] Patent Number:

5,851,832

[45] Date of Patent:

Dec. 22, 1998

[54] IN VITRO GROWTH AND PROLIFERATION OF MULTIPOTENT NEURAL STEM CELLS AND THEIR PROGENY

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[73] Assignee: Neurospheres, Ltd., Canada

[21] Appl. No.: 486,648

[22] Filed: Jun. 7, 1995

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 270,412, Jul. 5, 1994, abandoned, which is a continuation of Ser. No. 726,812, Jul. 8, 1991, abandoned, and a continuation-in-part of Ser. No. 385,404, Feb. 7, 1995, abandoned, which is a continuation of Ser. No. 961,813, Oct. 16, 1992, abandoned, which is a continuation-in-part of Ser. No. 725,812, and Ser. No. 359, 945, Dec. 20, 1994, abandoned, which is a continuation of Ser. No. 967,622, Oct. 28, 1992, abandoned, which is a continuation of Ser. No. 967,622, Oct. 28, 1992, abandoned, which is a continuation-in-part of Ser. No. 726,812, Jul. 8, 1991, abandoned, which is a continuation of Ser. No. 10,829, Jan. 29, 1993, abandoned, which is a continuation of Ser. No. 10,829, Jan. 29, 1993, abandoned, which is a continuation-in-part of Ser. No. 726,812, and Ser. No. 311,099, Sep. 23, 1994, abandoned, which is a continuation-in-part of Ser. No. 726,812, and Ser. No. 311,099, Sep. 23, 1994, abandoned, which is a continuation-in-part of Ser. No. 726,812, and Ser. No. 338, 730, Nov. 14, 1994, abandoned, which is a continuation-in-part of Ser. No. 726,812.

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[52]	U.S. Cl 435/368; 435/325; 435/366;
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[58]	Field of Search 435/240.2, 325,
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[56] References Cited

U.S. PATENT DOCUMENTS

4 753.635	6/1988	Sagen et al 604/49
4,980,174	12/1990	Sagen et al 424/563
5,082,670	1/1992	Gage 424/520
5,175,103	12/1992	Lee et al 435/172.3
5,411,883	5/1995	Boss et al 435/29
5,612,211	3/1997	Wilson et al 435/378

FOREIGN PATENT DOCUMENTS

8/1987	0 233 838
5/1989	89/03872
6/1990	90/06757
2/1991	91/02003
7/1991	91/09936
11/1991	91/17242
1/1993	93/01275
5/1993	93/09802
2/1994	94/03199
	5/1989 6/1990 2/1991 7/1991 11/1991 1/1993 5/1993

OTHER PUBLICATIONS

Almazan et al., "Epidermal Growth and Bovine Growth Hormone Stimulate Differentiation and Myelination of Brain Cell Aggregates in Culture," Developmental Brain Research, 21:257–264 (1985).

Anchan et al., "Trophic Factors Influence Proliferation of Germinal Neuroepithelial Cells of the Retina," *J. Cell Biol.*, 109:58a, Abstract No. 308 (1989).

Anchan et al., "EGF and TGF- α Stimulate Retinal Neuroepithelial Cell Proliferation in Vitro," Neuron, 6(6):923-936 (1991).

Bayer et al., "Neuron production in the Hippocampus and olfactory bulb of the adult rat Brain: addition or replacement?", Annals NY. Acad. Sci. 457:163–172 (1985).

Björklund et al., "Neural Grafting in Animal Models of Neurodegenerative Diseases," Ann. New York Acad. Sci., 457:53–81 (1985).

Bouvier et al., "Basic Fibroblast Growth Factor (bFGF) Promotes the Survival and Proliferation of Mesencephalic Neuronal Precursors in Vitro," Society for Neuroscience Abstracts, vol. 18, Abstract No.: 403.7 (1992).

Boyles et al., "Accumulation of Apolipoproteins in the Regenerating and Remyelinating Mammalian Peripheral Nerve," J. Biol. Chem., 265(29):17805–17815 (1990).

Calof et al., "Analysis of Neurogenesis in a Mammalian Neuroepithelium: Proliferation and Differentiation of an Olfactory Neuron Precursor in Vitro," *Neuron*, 3:115–127 (1989).

Cattaneo et al., "Identifying and Manipulating neuronal stem cells," TINS, 14(8): 338-340 (1991).

Cattaneo et al., "Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor," *Nature*, 347:762-765 (1990).

Cepko "Immortalization of neural cells via retrovirus-mediated oncogene transduction," Ann. Rev. Neurosci., 12:47-65 (1989).

Deloulme et al., "Establishment of Pure Neuronal Cultures From Fetal Rat Spinal Cord and Proliferation of the Neuronal Precursor Cells in the Presence of Fibroblast Growth Factor," *Journal of Neuroscience Research*, 29:499–509 (1991).

Dunnett et al., "Dopamine-rich transplants in experimental Parkinsonism," TINS, 266-270 (Jul. 1983).

Emerich et al., "Behavioral Effects of Neural Transplantation," Cell Transplantation, 1:1-27 (1992).

Faaland et al., "Rapid uptake of tyrphostin into A431 human epidermoid cells is followed by delayed inhibition of epidermal growth factor (EGF)-stimulated EGF receptor tyrosine kinase activity", Mol. Cell Biol. 11(5):2697-2703 (1991).

(List continued on next page.)

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Herbert LLP

[57] ABSTRACT

A method for the in vitro proliferation and differentiation of neural stem cells and stem cell progeny comprising the steps of (a) isolating the cells from a mammal, (b) exposing the cells to a culture medium containing a growth factor, (c) inducing the cells to proliferate, and (d) inducing the cells to differentiate is provided.

80 Claims, 3 Drawing Sheets